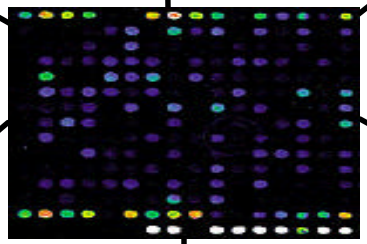
Metrology and Standards Needs for Gene Expression Technologies

Universal RNA Standards

March 28-29, 2003

Variations in Data Quality ?



Sample Preparation
- mRNA Extraction
-RNA Concentration
- Sample Contamination
- Signal Amplification
- Sample Purification

Array Fabrication
- Pen Shifts
- Sub-Array Variations
- Binding Chemistry
- Plate Contamination
- Depletion

Labeling
- Unbalanced Channels
- Dyes & Reagents
- Primer Incorporation
- Labeling Efficiency

Probe
- Sequence Verification
- Probe Length & Sequence
- Sequence Homology
- Oligo/Clone Specific Noise
- Cross-Reactivity

Scanning
- Scanner Settings
- Optical Measurements
- Overlap of channels
- Image Orientation

Hybridization
Hybridization Conditions
- Hybridization Gradients
- Amount of Sample Applied
- Cross-Reactivity
- Hybridization Efficiency

Goals of the Workshop

- **Educational:** provide participants a forum to share various methods and techniques that are relevant in defining a standard for Gene Expression and RT-PCR technologies
- **Awareness:** to determine areas of agreement and disagreement on issues of different standards,and where there is a need for additional information
- **Direction:** Will a single standard be capable enough to fulfill all our desired needs?
- **Guidance:**to gather input on how NISTcould best help to develop the RNA standard and promote its use .
- **Deliverable:**publish a workshop proceedings with the final outcomes of this workshop

Session 1
Standardization of Biological Component of RNA Based Molecular Assays

- **Focus:** The need for gene expression measurement standards to address the data requirements in support of:
  - Safety and efficacy claims of therapeutic products
  - Toxicity Evaluation
  - Human clinical in vitro diagnostics
- **Session chair: Frank Sistare, CDER, FDA**

Session 2
Metrics for Universal Standard: Expression Arrays

- **Metrics for evaluating sample and experimental variability**

  Quality (integrity/purity) of starting material
  Quality of processed (labeled/amplified) sample
  Hybridization performance (intensity, sensitivity, specificity)

- **Metrics for evaluating platform variability**

  Identification of array defects, quality control of substrate
  Integrity of feature location, quality control of probe manufacture

- **Image scanning variation/limitations**

- **<u>Session chair</u>: Janet Warrington, Affymetrix, Inc.**

Session 3
Defining the Universal RNA Standard

a. Allow performance validation of any single platform over time
b. Facilitate comparison between various platforms used to assay gene expression
c. Be constructed in such a manner as to assure consistency over time
d. Include two or more samples that allow one to make both absolute and relative measurements of the abundance of individual transcripts.
e. Not be limited to hybridization-based approaches, but should be amenable to use with other assays such as QRT-PCR

Session chair: **John Quackenbush, TIGR**

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- What are the key functions and features of standards for RT-PCR?

- What are currently implemented intra-laboratory controls and their effectiveness?

- **Session chair**: **John Sninsky, Celera Diagnostics**

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